REMARKS

The Office Action of April 7, 2004 presents the examination of claims 27-47. The claims are not further amended by this paper.

Rejection under 35 USC § 112, first paragraph

Claims 27-47 stand rejected under 35 USC § 112, first paragraph, for alleged lack of enablement. This rejection is respectfully traversed, reconsideration and withdrawal thereof are requested.

First, the Examiner fails to establish *prima facie* lack of enablement of the invention. "Enablement" of the invention is a matter of whether or not undue experimentation is required to practice the invention throughout its recited scope. The various factors that are to be weighed in examining this question are set forth in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). The Examiner does not undertake any detailed analysis of the question of undue experimentation, but only asserts (incorrectly) that there are no working examples in the specification and then asks how one of skill in the art can know whether a positive result from the screening assays set forth in the specification is, "due to antibody against *Mycobacteria*?" Such an argument is not sufficient to properly establish *prima facie* lack of enablement and so the rejection should be withdrawn.

Applicants further provide the following analysis of the question of undue experimentation.

Nature of the invention and hreadth of the claims

The invention lies in the field of polypeptide compositions having *inter alia* immunodiagnostic utility. The claims are of intermediate scope, being directed in their most broad embodiment to polypeptides comprising 4 specifically recited sequences or comprising sequences at least 70% identical thereto (claim 27). Additional claims are directed to polypeptides comprising specifically 5 other recited sequences (claims 28, etc.) The polypeptides of the invention are further described as being reactive with certain proportions of sera taken from populations infected with *Mycobacteria* and from uninfected populations.

The level of ordinary skill in the art

The level of ordinary skill in the art of peptide biochemistry and immunodiagnostics is generally considered to be high. The typical inventor in this art has a Ph.D. and/or M.D. degree and is familiar with the design and conduct of routine immunoassays for characterizing polypeptides.

The teachings of the specification and

the presence of working examples

The specification contains considerable disclosure related to how to make and use the claimed polypeptides. A generic strategy for obtaining polypeptides of the invention by expression of PCR products of genomic DNA of *Mycobacteria* is shown in flow-chart form in Figure 1. The Examiner should take note that the PCR product is chosen in the example of Figure 1 to include a "6X His" portion, for ease of purification of the expression product, appended to the portion encoding a *Mycobacterium* antigen. Figure 1 thus describes at least one embodiment of a polypeptide "comprising" a desired amino acid sequence.

Further purification of the expressed proteins by gel electrophoresis and preliminary characterization by N-terminal sequencing are described in Figure 2. Results of implementation of the strategy shown in Figures 1 and 2, and demonstration of differential reactivity with sera from patients with inactive *M. tuberculosis* infections and with sera from patients with active *M. tuberculosis* infections are shown in Figure 8, together with results using the same sera and a commercial TB diagnostic kit. (See, the figures per se and their description at pp. 5-6 of the specification.)

Additional species of *Mycobacterium* that might be used as sources of genomic DNA for implementing the strategy outlined above

are listed at page 8, lines 17-27. The skilled artisan would expect protein antigens derived from these species to have some sequence variation from the sequences expressly recited in the present claims and so a method for making variants of those sequences is disclosed. The Examiner might also note the sequence comparisons shown in Figure 3 between antigens defined by the sequence identifiers in the claims and known *M. tuberculosis* antigens. Algorithms for comparing sequences are described at page 11, lines 12-26 of the specification.

Various procedures for immunoassay are described at page 22, line 10 to page 24, line 19 and at page 25, lines 11-28. The latter portion describing the assay used in screening polypeptides of the invention for usefulness as diagnostic reagents as described in the figures. Yet further embodiments of these assays are described at page 20 of the specification.

Working examples of the isolation of polypeptides of the invention are provided at pp. 29-36 and in particular, the identification of polypeptides having differential reactivity with sera from infected patients with active infections, with sera from infected patients having inactive infections and uninfected patients is described in Examples 7 and 8 at pages 33-36 with results shown in Figures 4-6. An assay using combinations of antigens is described in Example 8 with results shown in Figure 9.

The state of the prior art

The reason the Examiner gives for why the instant invention is not enabled is that, in his personal opinion, and it is only that, one of skill in the art is not able to know with certainty that observation of a differential reactivity of human sera from infected vs. uninfected patients is due to the presence or absence from the sera of antibodies that bind to the portion of a polypeptide "comprising" a recited sequence, or variant thereof, rather than to some other portion of the polypeptide that is appended to such sequence. Notwithstanding that merely raising this question is not sufficient to establish *prima facie* non-enablement of the invention, Applicants submit that the state of the art at the time the invention was made was such that the skilled artisan could determine what portion of a polypeptide is responsible for binding to a polyclonal antiserum. As evidence that such is the case, Applicants attach hereto Exhibit 1, a collection of three abstracts, each of papers published before the filing date of the instant application, that describe mapping of the epitopes of polypeptide antigens that are bound by polyclonal antisera.

Applicants submit that Exhibit 1 establishes that the skilled artisan had at his disposal, as of the filing date of the application, the methods for determining whether a polypeptide operable in the present invention was useful because the antisera

of patients bound to (or did not bind to) one or more epitopes presented by a portion of the polypeptide representing a protein of a *Mycobacterium*.

The quantity of experimentation necessary

The amount of experimentation necessary to determine if a polypeptide of any given amino acid sequence is useful in the invention is modest. Exactly such an experiment is described in Examples 7 and 8 of the specification. The most difficult part of the experiments is perhaps the accumulation of serum samples from a substantial population of patients that are infected with *Mycobacterium* and that have active and inactive infections, and from a group of patients that are uninfected. As described at the bottom of page 33, about 85 total samples were used. Perhaps somewhat fewer samples could be collected, but in any event, as the inventors found, collecting 85 serum samples is not an insurmountable task.

The cloning of a PCR library into an expression vector and purification of the expressed proteins also does not require much experimentation and is in fact routine. The Examiner should note that the process described in Figure 1 makes use of commercially available kits for the cloning step. Purification of "his-tagged" proteins by nickel-NTA affinity chromatography is also a routine

method. In fact, all of the methods used in the screening process are routine biochemistry.

Furthermore, that a polypeptide should be screened to determine its operability in the present invention is experimentation that is expected to be performed by the skilled artisan. Experimentation that is expected is not undue, especially when the methods used are routine. *See, Wands.*

The predictability of the art

The predictability of the art is low in one aspect, but high in others. It is of course unpredictable whether a polypeptide of a completely arbitrary sequence would be operable in the present invention. However, to the degree that the polypeptide contains a sequence that is the same as one of those recited in the claims, it is rather predictable that it will present an epitope allowing differential binding to sera from patients actively infected with *Mycobacteria* compared to sera from patients inactively infected or uninfected. Furthermore, the specification provides sequences that are known to provide such differential binding, and computational comparison of a new sequence to these known sequences provides a prediction of whether the unknown sequence would be operable in the invention.

Furthermore, the issue is not whether it is predictable *a priori* a given sequence will have the properties recited in the

claims. Rather, the issue is whether it is predictable whether one of skill in the art, applying the teachings of the specification and of the art at the time the invention was made, can make embodiments in addition to those already explicitly described in the specification. Applicants submit that it is quite predictable that such can be accomplished.

On the whole, Applicants submit that a proper consideration of the factors to be weighed in evaluating enablement shows that no undue experimentation is required to practice the instantly claimed invention throughout its scope. Accordingly, the instant rejection should be withdrawn.

Rejection under 35 USC § 112, second paragraph

Claims 27-47 stand rejected under 35 USC § 112, second paragraph, for alleged lack of clarity. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

The Examiner asserts that the percentage reactivity is an indefinite term because it, "is totally dependent upon the number of subjects being tested." Applicants submit that, to the contrary, the term is quite definite as a numerical range is stated and further the claims relate to a population of subjects. Still further, the specification makes clear, *e.g.* by the working example

7, what is a representative sample size for determining the range stated in the claims.

Accordingly, the instant rejection should be withdrawn.

The present application well-describes and claims patentable subject matter. The favorable action of allowance of the pending claims and passage of the application to issue is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell (Reg. No. 36,623) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), Applicants respectfully petition for a two (2) month extension of time for filing a response in connection with the present application. The required fee of $420.00 is being filed concurrently with the Notice of Appeal.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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By _____
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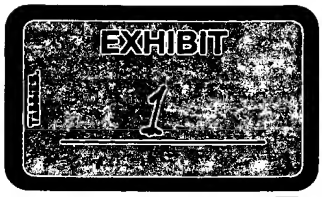
Attachment(s): Exhibit 1



1: Vet Immunol Immunopathol. 1999 Sep 1;70(1-2):117-24.

Domain mapping and comparative binding features of eight dog IgE-specific reagents in ELISA, immunoblots, and immunohistochemistry.

Griot-Wenk ME, Marti E, DeBoer DJ, de Weck AL, Lazary S.

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Eight dog IgE-specific reagents including monoclonal and polyclonal antibodies (Ab) and a cross-reactive alpha chain of the human high affinity IgE receptor were mapped to recombinant fragments of the second (IgEf2) and third/fourth (IgEf3/4) domains of the dog IgE heavy chain. In ELISA, five out of eight reagents reacted to solid-phase bound IgEf2, of which two polyclonal Ab bound in addition to IgEf3/4. All Ab which recognized at least one recombinant IgE fragment, also bound to IgE in ELISA, immunoblots, and immunohistochemistry. In contrast, only one monoclonal Ab, that did not bind to the recombinant IgE fragments, reacted with immunoblots of serum and immunohistochemistry. The alpha chain could only be applied to ELISA with serum IgE. Furthermore, there was a wide range of heat-lability of

binding reactions. Comparative analysis of available dog IgE-specific reagents enables more in-depth functional studies on IgE-mediated phenomena in dogs, and helps to further establish the dog as an animal model for allergy research.

PMID: 10507292 [PubMed - indexed for MEDLINE]

1: Protein Sci. 1999 Apr;8(4):760-70. Related Articles, Links

Actin surface structure revealed by antibody imprints: evaluation of phage-display analysis of anti-actin antibodies.

Jesaitis AJ, Gizachew D, Dratz EA, Siemsen DW, Stone KC, Burritt JB.

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Phage-display peptide library analysis of an anti-F actin polyclonal antibody identified 12 amino acid residues of actin that appear, in its X-ray crystal structure, to be grouped together in a surface accessible conformational epitope. Phage epitope mapping was carried out by isolating immune complexes containing members of the J404 nonapeptide phage-display library formed in diluted antiserum and isolated on a protein A affinity matrix. Immunoreactive clones were grown as plaques, replica plated onto nitrocellulose, and labeled with anti-actin immune serum. One hundred and forty-four positively staining clones identified in this way were sequenced. Of these, 54 displayed peptides with sequence similarities. When the most abundantly selected sequence, KQTWQQLWD, was

produced as a synthetic peptide and derivatized to ovalbumin, the complex was strongly recognized by the antiserum on Western blots and inhibited the binding of the antibody to immobilized F-actin by 60%. A scrambled version of this sequence WQDK WLQTQ, when coupled to ovalbumin, was not recognized by the antiserum and minimally inhibited binding of antiserum to immobilized F-actin by 10%. KQTWQQLWD contained four residues that corresponded, in frame, to a highly conserved six residue region of the chicken beta-actin sequence 351TFQQMW356 (identical residues are shown in bold). Examination of the rabbit skeletal muscle X-ray crystal structure suggested that within a 15 A radius of W356, nine additional residues were arranged on the actin surface in such a way that they could be mimicked by several of the selected phage sequences with root-mean-square deviation fits of 2.1-2.5 A. We conclude that phage-display analysis can provide information about the relative location of amino acids on the surfaces of proteins using antibody imprints of the protein surface structure.

PMID: 10211822 [PubMed - indexed for MEDLINE]

1: Dis Aquat Organ. 1998 Nov 30;34(3):167-76.

Mapping of linear antibody epitopes of the glycoprotein of VHSV, a salmonid rhabdovirus.

Fernandez-Alonso M, Lorenzo G, Perez L, Bullido R, Estepa A, Lorenzen N, Coll JM.

INIA, Sanidad Animal, CISA-Valdeolmos, Madrid, Spain.

Antibody linear epitopes of the glycoprotein G (gpG) of the viral haemorrhagic septicaemia virus (VHSV), a rhabdovirus of salmonids, were mapped by pepscan using overlapping 15-mer peptides covering the entire gpG sequence and ELISA with polyclonal and monoclonal murine and polyclonal trout antibodies. Among the regions recognized in the pepscan by the polyclonal antibodies (PAbs) were the previously identified phosphatidylserine binding heptad-repeats (Estepa & Coll 1996; Virology 216:60-70) and leucocyte stimulating peptides (Lorenzo et al. 1995; Virology 212:348-355). Among 17 monoclonal antibodies (MAbs), only 2 non-neutralizing MAbs, 110 (aa 139-153) and IP1H3 (aa 399-413), could be mapped to specific peptides in the pepscan of the gpG. Mapping of these MAbs was confirmed by immunoblotting with recombinant proteins and/or other synthetic peptides covering those sequences.

None of the neutralizing MAbs tested reacted with any of the gpG peptides. Previously mapped MAb resistant mutants in the gpG did not coincide with any of the linear epitopes defined by the pepscan strategy, suggesting the complementarity of the 2 methods for the identification of antibody recognition sites.

PMID: 9891732 [PubMed - indexed for MEDLINE]
